other indicator substances,

e) selecting the microorganisms which show an alteration in the substrate specificity,

said microorganism in steps b), d) and e) being a member selected from the group consisting of bacteria, fungi and yeasts.

Claim 2, line 2, delete "returning" and substitute --reisolating and retransforming--.

Cancel claim 3.

Claim 4, line 1, after "wherein" insert --the bacteria are--;

lines 2 and 3, delete ", fungi or yeasts are used as microorganisms--.

Claim 6, line 1, after "wherein" insert --the enzyme is--.

REMARKS

1. Regarding the IDS, applicants submit that the Winnacker reference *is* clear in its citation and should be considered. The reference is from From Genes to Clones, published in 1987 by VCH (publisher), and pages 126-127 contain the relevant information. The examiner is asked to specifically identify what is lacking in the citation.

Applicants will forward a copy of the Harpes reference, or the most relevant pages therefrom, as soon as it can be secured.

2. The examiner has objected to the specification for the reasons set forth in paragraph 2 of the office action. Applicants have made the following changes to the specification and care has been taken to avoid the introduction of new matter.

A. The title of the invention has been capitalized.

B. The term "regio-, chemo-, or stereoselective or" has been deleted from page 4, lines 25-26.

C. "PFE" is defined on page 11, line 17, as the esterase gene estF. The specification at p. 11, l. 17, has been amended to clarify this.

D. Page 13, line 4, of the specification has been amended to clarify that the overnight culture is one from E. Coli JM109 or DH5α which harbors the plasmid 2792.1, as described on page 11, lines 13-14.

E. Page 16, line 14, has been amended to indicate that the esterase is one which is expressed by clone PFE-U3. Applicants submit that there is inconsistency in the use of PFE-U1 on page 14 of the specification, as the specification describes that the mutation is not in the structural gene (page 14, lines 31-33).

F. A brief description of the drawings has been inserted at page 3, line 41, of the specification.

3. The examiner has objected to claim 3 as failing to further limit the claim upon which it depends (claim 1). Claim 3 has been canceled and the microorganism recited in steps c) to e) of claim 1 is further defined therein as a member selected from bacteria, fungi and yeasts. In accordance therewith, claim 4 has been amended to further define the bacteria recited in claim 1.

4. Claims 1, 2, 6 and 7 have been rejected under 35 USC 112, second paragraph, as

being indefinite. Specifically, the examiner has objected to the terms "transmitting" (claim 1) and returning the DNA" (claim 2). To obviate this rejection, applicants have amended the term "transmitting" in claim 1 to read "transferring", in accordance with the specification disclosure at page 6, line 21, and have changed "returning" in claim 2 to "reisolating and retransforming", in accordance with the specification at page 7, lines 23-26 and Fig. 1.

The examiner also objected to claims 6 and 7 as reciting improper Markush language. Applicants submit that the Markush language of claims 6 and 7 is indeed proper, as all of the enzymes mentioned in these two claims are hydrolases (see specification at page 4, lines 9-18).

It is believed by applicants that claims 1, 2, 6 and 7 now particularly point out and distinctly claim the subject invention, in accordance with 35 USC 112, second paragraph. Favorable reconsideration of the rejection is thus urged.

5. Claims 1, and 4-8 have been rejected under 35 USC 102(b) as being anticipated by Greener et al. Applicants assert that the instant claims do not read on the disclosure of Greener, as the instant claims are directed to a method of altering the substrate specificity of an enzyme, something at which the Greener reference does not even hint. Greener describes a method of increasing the antibiotic resistance of *E. coli* XL-1-Red by increasing the copy number of the plasmid pBR322. The Greener method involves a site-directed mutagenesis of a phosphatase gene, a method which by necessity requires that detailed structural information about the protein be available (see p. 375, paragraph

1 of Greener). There is no mention or suggestion in Greener of a method for altering the substrate specificity of an enzyme by carrying out the steps of the instantly claimed invention.

The examiner states:

> Although Greener et al. does not specifically define an increase in specific activity as a change in substrate specificity, it is inherently so in lieu [sic] of both applicants' specification and the generally accepted use of the term "substrate specificity" in the art. Greener et al. conclude that "these mutations result [ ] in a variant having a higher specific activity...".

Clearly, the examiner has misquoted the Greener reference. What Greener actually states at page 384 is:

> Presumably, these structural gene mutations result in a variant having higher specific activity.. (emphasis supplied)

Thus, the Greener disclosure fails to state unequivocally that the mutagenesis described therein leads to an increase in specific acticvity of the enzyme, let alone any alteration in the substrate specificity. The examiner simply has not made out a *prima facie* case that the instant invention as claimed is disclosed by Greener.

In view of the above, applicants request that the rejection of claims 1-2 and 4-8 as anticipated by Greener be withdrawn.

Claims 1-9 have been rejected under 35 USC 103(a) as being unpatentable over Greener et al. in view of Wilks et al. The Greener reference has been discussed in the section above with regards to the Section 102 rejection. Applicants submit that a combination of the Greener and Wilks references would not have provided the necessary

motivation to have led a person of ordinary skill in the art to applicants' invention as claimed. Wilks et al. teaches the difficult reconstruction of a redesigned lactate dehydrogenase by replacing certain amino acids by others (see page 153, col. 1, paragraph). Wilks et al. teaches as follows: "Chemical intuition was then used to replace these (= amino acids) with other natural amino acid site chains to avoid the side chain clashes with the new substrate. Both the original native enzyme with pyruvate and the mutant ^{102,103,105}Gln, Lys, Pro, ^{235,236}Ala, Ala $\longrightarrow$ ^{102,103,105}Met, Val, Ser, ^{235,236}Gly, Gly were then minimized using Biosym Associates program Discover v. 2.5. This is a method which requires substantial information to modify the special enzyme and therefore is not a general method. Applicants' invention, on the other hand, does not require knowledge of such detailed information. There is no hint at applicants' invention, either in Wilks et al. or in Greener et al. or in the combination of the references for the skilled person of applicants' invention. Applicants submit that the examiner has adopted a forbidden hindsight approach in reaching a conclusion of obviousness. Favorable reconsideration of the rejection under 35 USC 103(a) is thus urged.

CONCLUSION

Based on the above amendment and remarks, applicants submit that the instant claims are in condition for allowance and an early notice to that end is solicited.

Please charge any shortage in fees due in connection with the filing of this paper,

including Extension of Time fees to Deposit Account No. 11-0345. Please credit any excess fees to such deposit account.

Respectfully submitted,

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